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(54) **Treatment of biological and pharmaceutical products adsorbed on a solid phase with virus and pyrogen inactivating agents.**

(57) A method for treating a biological or pharmaceutical product to inactivate viruses and pyrogens therein comprising the steps of adsorbing said product onto a solid phase; treating the adsorbed product with a virus or pyrogen inactivating agent; separating the solid phase and quantitatively removing the residual inactivating agent therefrom; and recovering said product.

TREATMENT OF BIOLOGICAL AND PHARMACEUTICAL PRODUCTS  
ADSORBED ON A SOLID PHASE WITH VIRUS AND PHYROGEN  
INACTIVATING AGENTS

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5 This invention relates to a method of reducing the pyrogenicity and infectivity of hepatitis B or other viruses in biological and pharmaceutical products. More particularly this invention relates to a method of in-activating viruses and pyrogens which contaminate biological and pharmaceutical products by adsorbing said products on a  
10 solid phase and subjecting them to a treatment with an agent which inactivates viruses and/or pyrogens.

It is well-known that biological and pharmaceutical products may contain transmissible biological contaminants,  
15 such as viruses, and especially infectious hepatitis viruses, such as hepatitis virus B, and non-A, non-B hepatitis viruses. Contamination by viruses may result from both the source material used and from the environment during their production.

Pyrogens are lipopolysaccharides also known as  
20 endotoxins, having numerous biologic activities which include the production of fever, activation of clotting mechanisms and induction of shock. Consequently, it is essential that pyrogenic substances be removed from products intended for biological or pharmaceutical use.

25 Methods for inactivation or destruction of viruses and/or pyrogens include treatment with heat, acid or alkali, filtration, removal by adsorption with gels, ion-exchange resin and various other such adsorbent material. Most of these methods are burdensome, time consuming, or destructive  
30 of the product due to the rigorousness of the treatment.

1           Simple treatment with virus or pyrogen inactivating  
agents like Triton, Tween, beta-propiolactone or hypochlorite  
may result in inactivation of the viruses and/or pyrogenic  
5           materials but is accompanied by partial denaturation of  
labile proteins. Even if the proteins are not denatured, the  
quantitative removal of the residual virus inactivating agent  
or pyrogen inactivating agent may be very difficult and its  
contamination makes the biological or pharmaceutical product  
undesirable.

10           Illustrative of the use of virus and/or pyrogen  
inactivating agents is U.S.P. No. 4,314,957 which discloses  
the use of amphiphiles to destroy endotoxin and to inactivate  
hepatitis viruses in plasma derivatives by direct contact of  
15           the amphiphiles with the plasma derivatives in solution.  
After treatment, the plasma proteins are separated from the  
amphiphiles by precipitation. Such separation may not  
completely remove the inactivating agents from the precipi-  
tated protein and the final product, therefore, may contain  
20           pharmaceutically harmful and/or undesirable inactivating  
agents.

1.           A method to depyrogenate or inactivate viruses in a  
biological or pharmaceutical product comprising the steps of:  
25           adsorbing said product onto a solid phase;  
              contacting said product adsorbed on said solid phase  
with a virus inactivating or depyrogenating agent;

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1 separating the solid phase from the virus inactivating  
or depyrogenating agent;

5 removing impurities and residual inactivating or  
depyrogenating agent from the solid phase; and  
recovering the product from the solid phase.

10 The process of the present invention is applicable  
to any material in the biomedical field intended to be used  
in the human or animal body for biomedical or therapeutic  
purposes as well as non-therapeutic experimental purposes.  
Contemplated biomedical products and materials which can be  
depyrogenated or made free of viruses using the process of  
15 the present invention include but are not limited to:

blood fractions such as antihemophilic factor  
A (AHF, Factor VIII), prothrombin complex  
(Factors II, VII, IX and X), individual or  
group of Factor II, Factor VII, Factor IX,

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1 Factor X, Protein C, Antithrombin III, C-1  
Esterase Inhibitor, Fibronectin, Gamma Globulin,  
and Albumin derived from human or animal origin;  
Biological and pharmaceutical products derived  
5 from animal origin, e.g. insulin, enzymes,  
coenzymes, antibodies, and hormones; and  
biological products derived from human or animal  
placentae, e.g., blood fractions, and vaccines.  
These products and materials are available from  
10 various commercial sources or can be produced by using well-  
known preparative techniques. For example, blood fractions  
and blood proteins can be obtained from human blood plasma  
by fractionation according to known techniques such as, for  
example, the alcohol fractionation method of Cohn described  
15 in U.S. Pat. No. 2,390,074 and the Journal of the American  
Chemical Society Vol. 68, p. 459 (1946). These methods as  
well as other techniques are summarized in "The Plasma  
Proteins", second edition, Volume III, pp. 548-550, Academic  
Press, New York, N.Y. (1977).

20 As used herein, the "solid phase" on which said  
biological or pharmaceutical product is adsorbed is meant  
to define a substance used as ion exchanger, resins used  
for affinity chromatography, resins with an attached anti-  
body against the particular or related antigen, special  
25 membrane media which as an ion exchanger or are capable of  
adsorbing particular substances, or glass beads which are  
treated to produce surfaces that act as an ion exchanger  
affinity resin. Contemplated solid phase materials include:  
a,  
30 An ion exchanger, e.g., DEAE Sephadex QAE  
Sephadex, CM-Sephadex, SP-Sephadex DEAE Biogel A,  
CM Biogel A, Biogel HTP, DEAE Cellulose:

- 1 b, Resins used for affinity chromatography, such  
as Sepharose 2B, Sepharose 4B, Sepharose 6B,  
5 Sepharose CL-2B, Sepharose CL-4B and Biogel  
A-15 with an attached ligand consisting of, for  
example, an inhibitor, enzyme, coenzyme, or  
hormone. The attached ligand is capable of  
adsorbing the biological or pharmaceutical  
product, for example, heparin attached to a  
10 sepharose resin adsorbs Antithrombin III. The  
attached ligand may be an antibody to the antigen  
or related antigen present in the product. An  
example is antibody to Antihemophilic Factor  
related antigen attached to a Sepharose resin to  
15 adsorb Antihemophilic Factor. The attached ligand  
may also be a monoclonal or polyclonal antibody  
to the antigen which might be desired to be isolated  
as the biological or pharmaceutical product;
- c, Resins described in b above with an attached anti-  
body (monoclonal or polyclonal) against a particular  
20 antigen or an antigen itself;
- d, Special membrane media which act as an ion exchanger,  
e.g., Zeta-Prep<sup>TM</sup> cartridge, DEAE, QAE, and SP;
- e, Controlled-Pore Glass beads which are treated to  
25 produce surfaces that act as an ion exchanger, e.g.,  
DEAE-CPG, CML-CPG; and
- f, Controlled-Pore Glass beads attached with biologicals  
of interest such as inhibitors, enzymes, coenzymes,  
hormones, antibodies, and antigens.

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1           The virus inactivating and depyrogenating agents  
used in the present invention are the amphiphiles, organic  
solvents, hypochlorite or beta propiolactone.

5           The term amphiphile means a substance containing  
both hydrophilic water soluble and hydrophobic water-insoluble  
groups and which are generally classified as cationic,  
anionic, ampholytic and non-ionic surface active agents.

          Agents of the cationic type include long chain  
amine condensates with ethylene oxide and quaternary ammonium  
10 compounds, for example cetyl trimethyl ammonium bromide and  
dodecyl dimethyl ammonium bromide. Suitable anionic agents  
include soaps, salts of aliphatic monoesters of sulphuric  
acid, for example sodium lauryl sulphate and sodium  
heptadecyl sulphate, sulphonated aromatic compounds, for  
15 example alkyl benzene sulphonic acids and salts thereof such  
as tridecylbenzene sulphonic acid and the sodium and amino  
salts of dodecylbenzene sulphonic acid, alkyl naphthalene  
sulphonates such as sodium butyl naphthalene sulphonate,  
sulphosuccinates such as sodium dioctyl sulphosuccinate, and  
20 N-acyl-N-alkyl fatty acid taurates.

          Non-ionic agents include (a) ethoxylated  
alkylphenols (b) ethoxylated aliphatic alcohols, (c)  
carboxylic esters and (d) carboxylic amides, as described  
hereinafter

25 (a)   The ethoxylated alkylphenol non-ionic surface active  
agents include various polyethylene oxide condensates of  
alkylphenols, especially the condensation products of  
mono-alkylphenols or di-alkylphenols wherein the alkyl  
group contains about 6 to about 12 carbon atoms in either  
30 branched chain or particularly straight chain configuration,  
for example octyl cresol, octyl phenol or nonyl phenol, with

1 ethylene oxide, the said ethylene oxide being  
present in amounts equal to from about 5 to about 25  
moles of ethylene oxide per mole of alkylphenol.

(b) One particular type of ethoxylated aliphatic  
5 alcohol non-ionic surface active agents is the condensation  
products of aliphatic alcohols having from about 8 to 18 carbon  
atoms in either straight chain or branched chain configura-  
tion, for example oleyl or cetyl alcohol, with ethylene  
oxide, the said ethylene oxide being present in equal  
10 amounts of from about 30 to about 60 moles of ethylene  
oxide per mole of alcohol.

(c) Particular types of carboxylic ester non-ionic  
surface active agents are firstly the partial, for example  
mono-esters formed by the reaction of fatty and resin  
15 acids, for example of about 8 to about 18 carbon atoms  
with polyhydric alcohols, for example glycerol, glycols  
such as mono-, di-, tetra- and hexaethylene glycol,  
sorbitan, etc; and similar compounds formed by the direct  
addition of varying molar ratios of ethylene oxide to the  
20 hydroxy group of fatty acids.

A second type of carboxylic esters is the  
condensation products of fatty and resin acid partial, for  
example mono-esters with ethylene oxide, such as fatty or  
resin acid esters of polyoxyethylene sorbitan and sorbitol,  
25 for example polyoxyethylene sorbitan mono-tall oil esters.  
These may contain, for example, from about 3 to about 80  
oxyethylene units per molecule and fatty or resin acid  
groups of from about 8 to about 18 carbon atoms. Examples  
of naturally occurring fatty acid mixtures which may be  
30 used are those from coconut oil and tallow whilst examples  
of single fatty acids are dodecanoic acid and oleic acid.



1 (d) One particular type of carboxylic amide non-ionic  
surface active agents is the ammonia, monoethanol and  
diethanol amides of fatty acids having an acyl chain of  
from about 8 to about 18 carbon atoms.

5 The ampholitic agents include dodecyl -alanine,  
N-dodecylaminoethanesulfonic acid, palmitoyllysolecithin  
and dodecyl-N-betaine.

Organic solvents which may be used for the process  
of the present invention include dimethyl ether, diethyl  
10 ether, ethyl propyl ether, methyl-butyl ether, methyl isopropyl  
ether, methyl isobutyl ether, chloroform, methanol, ethanol,  
propanol, isopropanol, n-butanol, isobutanol, n-pentanol  
and ispentanols.

Referring now specifically to the process steps of  
15 the present invention, the product to be treated is first  
adsorbed onto the solid phase, followed by subjecting the  
so-adsorbed product to the virus or pyrogen inactivating  
agent in a liquid or gas phase for sufficient time to  
complete inactivation and depyrogenation therein. The virus  
20 or pyrogen inactivating agent is generally present in  
amounts of from 0.1% to 50%, preferably from 0.5% to 20%,  
and most preferably 1% to 10% based on the volume of the  
product. If the inactivating agent is in the liquid phase,  
the pH of the solution should be from about 5 to 9 and  
25 preferably from about 6 to 8. The time necessary to  
complete inactivation generally is from 2 minutes to 16  
hours, more preferably from 1 to 10 hours. The process of  
inactivation may be preformed at a temperature ranging from  
0°C to 50°C; if the inactivating agents used are gaseous,  
30 the temperature range is generally lower, while liquid  
phase inactivating agents are employed at or close to room

1 temperature. The inactivating agent in gaseous form  
can be used as such or as an aerosol spray to treat the  
solid phase adsorbed product.

5 Preferably, the treatment of the biomedical or  
pharmaceutical product adsorbed on a solid phase is  
carried out with the virus or pyrogen inactivating agent,  
by treating the solid phase adsorbed product with a buffer  
solution containing an inactivating agent. The treatment  
is carried out in a column operation or batchwise by  
10 immersing or soaking the solid phase in the buffer solution  
containing the inactivating agent. Suitable buffer  
solutions include: phosphate, citrate, tris aminomethane,  
glycine or any other buffering agent which does not adversely  
interact with the biological or pharmaceutical material  
15 being processed.

Upon completing the inactivation step, the in-  
activating agent is removed by separating the liquid or gas  
phase from the solid phase. The liquid phase can be  
separated by the use of a column, by centrifuging or by  
20 filtration.

Upon completing the separation, the solid phase  
is washed extensively with a solution of buffer used in  
the previous step of inactivation, said buffer having an  
ionic strength of 0.05M to 2.0M, and preferably 0.10M to  
25 0.25M, and a pH of from about 5 to 9, and preferably from  
about 6 to 8. The washing is to remove impurities adsorbed  
on the solid phase and to remove the agent used to  
depyrogenate or inactivate the virus. This is preferably  
accomplished in a column or batchwise operation. While a  
30 solution of the same buffering agent is preferred to remove  
impurities and quantitatively remove the inactivating agent,

1 other solutions such as phosphate, citrate, tris,  
glycine or any buffering agent which does not interact  
with the biological or pharmaceutical material and has  
similar ionic strength may also be used.

5           The pure biological or pharmaceutical product  
is then eluted from the solid phase with a buffer  
solution of phosphate, citrate, tris aminomethane, glycine  
or any other buffering agent which does not adversely  
interact with the biological or pharmaceutical material  
10 being processed and has an ionic strength of 0.15M to  
3.5M, and preferably from 0.5M to 2.5M, and a pH of from  
about 5 to 9, and preferably from about 6 to 8. Other  
solutions of sodium chloride, calcium chloride, magnesium  
chloride, epsilon amino caproic acid, or sodium citrate in  
15 a buffer described above may also be used for eluting the  
pure product.

In order to more fully illustrate the nature of  
the invention and the manner of practicing the same, the  
following examples are presented:

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EXAMPLE 1

Human plasma fraction, cryopoor plasma, used for Prothrombin Complex isolation, is adsorbed on DEAE-Sephadex A-50 (Pharmacia). The ion exchanger with  
5 Prothrombin Complex proteins adsorbed on it is then treated with Triton X-100 radio labelled with Triton (2% Triton X-100 containing  $^3\text{H}$  radio labelled Triton X-100 which gives a total of 167,340 counts per minute). Triton X-100 solution is then separated by filtration and the DEAE-  
10 Sephadex is washed three times with a buffer solution (0.01M sodium citrate, 0.2M sodium chloride, pH 7.0). During these washings all the Triton X-100 is washed off. Thereafter, Prothrombin Complex is eluted by a buffer of higher ionic strength (0.01M sodium citrate, 2.0M sodium chloride, pH 7.0).  
15 This eluate is found to be free of Triton X-100 as shown in Table I. (As seen in Table I, more than 99.99% of the agent is removed.) Thus the agent used to inactivate viruses or pyrogens is quantitatively removed and the final product is free of the agent.

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TABLE I

|  | TOTAL CPM   | % OF ORIGINAL<br>RADIOACTIVITY |
|--|-------------|--------------------------------|
| TRITON X-100 CONTAINING <sup>3</sup> H TRITON<br>X-100 ADDED DURING 1ST WASH | 167,340,000 | 100                            |
| 1ST WASH   | 162,470,000 |                                |
| 2ND WASH   | 4,350,500   | 99.93                          |
| 3RD WASH   | 507,500     |                                |
| ELUATE   | 11,640      | 0.0069                         |

EXAMPLE 2

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Plasma fraction used for Prothrombin Complex isolation (plasma from which cryoprecipitate was removed) is spiked with Sindbis or Vesicular Stomatitis virus (VSV). It is then adsorbed on DEAE-Sephadex and treated with 2% Triton X-100 solution for 30 minutes to inactivate the virus. The ion exchanger is then separated from Triton X-100 solution by filtration and washed three times with a buffer consisting of 0.01M sodium citrate and 0.2M sodium chloride, at pH 7.0, to wash off residual Triton X-100 and impurities bound to DEAE-Sephadex non-specifically. The Prothrombin Complex is eluted by a buffer of higher ionic strength (2M sodium chloride, 0.01M sodium citrate, at pH 7.0), diafiltered, and ultrafiltered to physiological conditions. As control, experiments are repeated after spiking the plasma fractions with the respective virus. However, treatment of Prothrombin Complex after adsorption on DEAE-Sephadex with 2% Triton X-100 is omitted in the control experiment.

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The final Prothrombin Complex, the starting plasma fractions, and intermediate fractions were assayed for virus activity by a method similar to that described by E. R. Pfefferkon and H. S. Hunter (Virology 20, 433-445, 1963), and briefly summarized hereunder.

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Chick embryo fibroblasts (CEF) were seeded into 100mm plastic dishes and allowed to grow to confluence (48 Hours). Serial tenfold dilutions of samples were made in minimal essential medium (MEM) containing 10% fetal calf serum and 1.0 ml inoculated onto replicate plates from which culture medium had previously been drained. The plates were incubated for one hour at 37°C in 5% CO<sub>2</sub> after which the inoculum was aspirated and 10 ml per plate of overlay medium added. The overlay medium consisted of MEM with 10%

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1 heat inactivated fetal calf serum and 1% agarose. The overlay was allowed to solidify after which cultures were incubated at 37°C in 5% CO<sub>2</sub> for about 24 hours. At this time, cytopathology was evident in positive cultures.

5 A 0.002% solution of neutral red in phosphate buffered saline was then added, and cultures were incubated an additional four hours. After this period, plaques were counted and virus activity was represented in terms of plaque forming units (PFU).

10 Table II and III show that by treatment with Triton X-100, using the method described in this invention, virus activities of Sindbis and VSV are reduced  $2.07 \times 10^3$  and  $8.45 \times 10^4$  fold respectively as compared to control experiments.

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TABLE II  
SINDBIS INACTIVATION DURING PURIFICATION AND  
DETERGENT TREATMENT

|   | TRITON TREATMENT        |                        |                       | CONTROL                 |                        |                       |
|---|-------------------------|------------------------|-----------------------|-------------------------|------------------------|-----------------------|
|   | VOL-ML                  | VIRUS AMOUNT<br>PFU/ML | TOTAL PFU             | VOL-ML                  | VIRUS AMOUNT<br>PFU/ML | TOTAL PFU             |
| STARTING PLASMA FRACTION                | 10,000                  | $7.76 \times 10^6$     | $7.76 \times 10^{10}$ | 10,000                  | $1.15 \times 10^7$     | $1.15 \times 10^{11}$ |
| DEAF-UNADSORBED                         | 10,000                  | $6.10 \times 10^6$     | $6.10 \times 10^{10}$ | 10,000                  | $5.95 \times 10^6$     | $5.95 \times 10^{10}$ |
| WASH (TRITON)                           | 500                     | -                      | -                     | -                       | -                      | -                     |
| WASH - 1                                | 3,340                   | $3.90 \times 10^2$     | $1.30 \times 10^6$    | 3,340                   | $5.03 \times 10^5$     | $1.95 \times 10^8$    |
| WASH - 2                                | 3,340                   | -                      | -                     | 3,340                   | -                      | -                     |
| WASH - 3                                | 3,340                   | -                      | -                     | 3,340                   | -                      | -                     |
| ELUATE                                  | 200                     | $9.83 \times 10^2$     | $1.97 \times 10^5$    | 220                     | $6.83 \times 10^6$     | $1.50 \times 10^9$    |
| PROTHROMBIN COMPLEX BULK<br>AFTER UP/DF | 152                     | $2.24 \times 10^3$     | $3.40 \times 10^5$    | 201                     | $5.22 \times 10^6$     | $1.04 \times 10^9$    |
| REDUCTION IN VIRUS                      | $2.28 \times 10^5$ FOLD |                        |                       | $1.10 \times 10^2$ FOLD |                        |                       |
| REDUCTION AS COMPARED<br>TO CONTROL     | $2.07 \times 10^3$ FOLD |                        |                       |                         |                        |                       |



TABLE III  
VSV INACTIVATION DURING PURIFICATION AND  
DETERGENT TREATMENT

|   | TRITON TREATMENT          |                   |                           | CONTROL |                    |                           |
|---|---------------------------|-------------------|---------------------------|---------|--------------------|---------------------------|
|   | VOL-ML                    | PFU/ML            | VIRUS AMOUNT<br>TOTAL PFU | VOL-ML  | PFU/ML             | VIRUS AMOUNT<br>TOTAL PFU |
| STARTING PLASMA FRACTION                | 10,000                    | $5.0 \times 10^3$ | $5.0 \times 10^7$         | 10,000  | $4.5 \times 10^3$  | $4.5 \times 10^7$         |
| DEAE-UNADSORBED                         | 10,000                    | $1.5 \times 10^3$ | $1.5 \times 10^7$         | 10,000  | $3.0 \times 10^3$  | $3.0 \times 10^7$         |
| WASH (TRITON)                           | 500                       | -                 | -                         | -       | -                  | -                         |
| WASH - 1                                | 3,340                     | -                 | -                         | 3,340   | $4.0 \times 10^2$  | $1.36 \times 10^6$        |
| WASH - 2                                | 3,340                     | -                 | -                         | 3,340   | -                  | -                         |
| WASH - 3                                | 3,340                     | -                 | -                         | 3,340   | -                  | -                         |
| ELUATE                                  | 300                       | $< 0.25$          | $< 7.5 \times 10^1$       | 300     | $1.93 \times 10^4$ | $5.79 \times 10^6$        |
| PROTHROMBIN COMPLEX BULK<br>AFTER UF/DF | 216                       | $< 0.25$          | $< 5.4 \times 10^1$       | 212     | $2.91 \times 10^4$ | $6.17 \times 10^6$        |
| REDUCTION IN VIRUS                      | $> 9.3 \times 10^5$ FOLD  |                   |                           | 11 FOLD |                    |                           |
| REDUCTION AS COMPARED<br>TO CONTROL     | $> 8.45 \times 10^4$ FOLD |                   |                           |         |                    |                           |

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EXAMPLE 3

250 ml cryopoor plasma is spiked with endotoxin to obtain a concentration of 100 ng/ml. The spiked plasma is then stirred with 4 g. of preswollen DEAE-  
5 Sephadex to adsorb Prothrombin Complex factors including Factor IX on the resin. The resin is then treated with 2% Triton X-100 solution in 0.01M sodium citrate, 0.2M sodium chloride at pH 7.0 to depyrogenate. Thereafter, the resin is washed three times with aliquots of 85 ml  
10 solution of 0.01M sodium citrate, 0.2M sodium chloride at pH 7.0. Factor IX is then eluted from the resin by a solution of 0.01M sodium citrate, 2M sodium chloride at pH 7.0, dialyzed, and ultrafiltered against 0.13M sodium chloride, 0.01M sodium citrate at pH 7.0 to a volume of  
15 5 ml. In control experiment treatment of factor IX after adsorption on DEAE-Sephadex with Triton X-100 is omitted.

The final Prothrombin Complex concentrates thus obtained are assayed for Factor IX potency, clotting factors' activation, and endotoxin. Factor IX is assayed  
20 using a slight modification of the one stage method of Jarrow and Graham (In Tocantins and Kazel, Blood Coagulation, Hemorrhage and Thrombosis, Grune and Stratton, New York 1964 p. 120), activation measured by the Nonactivated Partial Thromboplastin Time Test (NAPTT) of Kingdon and coworkers  
25 (Thromb. Diath. Haemorrh. 33, 617-631, 1975) and endotoxin by a modification of LAL test described by Levin and co-workers (Ann. Intern. Med. 76:1, 1972).

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1           Results presented in Table IV show that treatment  
with 2% Triton X-100 using the method of this invention  
results in practically no impact on Factor IX potency,  
Prothrombin Complex factors remain in zymogen form (non-  
5 activated), and the level of endotoxin is reduced by 70 ng/ml.

TABLE IV

| 10 | TEST           | PROTHROMBIN COMPLEX CONCENTRATE |                      |
|----|----------------|---------------------------------|----------------------|
|    |                | CONTROL                         | TRITON X-100 TREATED |
|    | FACTOR IX U/ML | 23.2                            | 21.6                 |
| 15 | NAPTT          | Negative                        | Negative             |
|    | LAL NG/ML      | 80                              | 10                   |

Examples 1 - 3 show that by the use of DEAE-  
20 Sephadex resin as a solid phase and Triton X-100 as virus  
inactivating or depyrogenating agent, proteins of Prothrombin  
Complex, e.g., Factor IX, are depyrogenated and depleted of  
virus contamination and the end product results in having  
no residual amounts of Triton X-100.

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EXAMPLE 4

Depyrogenation of Plasminogen by Treatment with Triton X-100 on Lysine-Sapharose Solid Phase.

Plasma is spiked with endotoxin at a final  
5 concentration of 20 ng/ml, then it is applied on a  
column consisting of Lysine-Sepharose resin. Plasminogen  
is adsorbed to the column. In the column, resin is then  
treated with 2% Triton X-100 to depyrogenate it. Residual  
Triton is then removed by washing with 0.3M phosphate  
10 buffer at pH 7.3, and plasminogen is eluted by a solution  
of 0.2M epsilon amino caproic acid on 0.05M phosphate  
buffer at pH 7.3. Finally, the eluate is dialyzed against  
phosphate buffered saline solution at pH 7.3, and a  
purified preparation of plasminogen with less than 0.1 ng  
15 endotoxin per ml is obtained.

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EXAMPLE 5

Inactivation of Vesicular Stomatitis Virus (a marker)  
During Isolation of Antithrombin III by treatment on  
Heparin-Sepharose, Solid Phase.

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Heparin attached to Sepharose is packed in a  
column. Cryopoor plasma is spiked with VSV to a titer  
of  $5 \times 10^6$  total PFU and passed through this column.

Antithrombin III is adsorbed on the solid phase, which  
is then treated with 2% Triton X-100 in phosphate

10 buffered saline to inactivate the virus. The column is

then washed extensively with 0.5M sodium chloride in  
phosphate buffer, at pH 7.3, to remove residual Triton

X-100 and the impurities bound nonspecifically to the

solid phase. Thereafter, Antithrombin III is eluted by

15 2M sodium chloride in phosphate buffer at pH 7.3. The

Antithrombin III is then dialyzed against phosphate

buffered saline at pH 7.3 to obtain a final purified

Antithrombin III preparation in which the virus was

reduced to less than 0.25 PFU/ml.

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EXAMPLE 6

Inactivation of Viruses at the Solid Phase Consisting  
of Monoclonal Antibody Against Factor VIII:R Ligand  
Attached to Sepharose During Isolation of Factor VIII.

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Monoclonal antibody against Factor VIII:R Ag is  
attached to sepharose resin, and packed in a column. A  
suspension of cryoprecipitate is spiked with VSV, as a  
controlled experiment, to a concentration of approximately  
 $5 \times 10^7$  PFU. It is then passed through the column where

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Factor VIII molecules (which consist of subunits of Factor  
VIII:C Antigen and Factor VIII:R Antigen) adsorb through  
the Factor VIII:R Antigen portion of the molecule. The  
resin is then treated with 2% Tween 80 solution in a wash  
buffer consisting of 100mM lysine, 20mM histidine, 0.15M  
sodium chloride, at pH 7.0, to inactivate the virus.

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Residual Tween 80 is removed by an extensive washing of  
the column using the above wash buffer. The Factor VIII:C  
portion of the Factor VIII molecule is then desorbed by an  
elution buffer consisting of 0.25M calcium chloride in  
the wash buffer. Once Factor VIII:C antigen is desorbed,  
remaining Factor VIII:R antigen is also desorbed by 3M  
sodium thiocyanate in the above wash buffer. Both Factor  
VIII:C and Factor VIII:R solutions are separately concen-  
trated and dialyzed against normal physiological saline  
to obtain Factor VIII:C (clotting) and Factor VIII:R  
(von Willebrand) preparations practically free of virus,  
i.e., less than 0.25 PFU/ml.

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EXAMPLE 7

Isolation of Protein C free of Viruses by Treatment with Triton X-100 at a Solid Phase Consisting of Pore Glass CPG-DEAE.

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Cryopoor plasma spiked with a marker virus, e.g., VSV to a concentration of  $5 \times 10^6$  total PFU is passed through a column packed with CPG-DEAE. Protein C is adsorbed on the column which is then treated with 2% Triton X-100 in the wash buffer, 0.01M sodium citrate, 10 0.2M sodium chloride, at pH 7.0, to inactivate the marker virus. Residual Triton X-100 and other impurities are removed by washing the column extensively with wash buffer. Protein C is then eluted from the column by 0.25M sodium citrate, 0.55M sodium chloride at pH 6.0. The 15 eluate is then dialyzed against normal physiological saline solution and a Protein C preparation free of marker virus, VSV, (less than 0.25 PFU/ml) is obtained.

Various modifications will be apparent to the person skilled in the art after reading the present 20 disclosure without departing from the spirit and scope of the invention; it is intended that all such modifications be included within the scope of the appended claims.

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## 1 WHAT IS CLAIMED IS:

1. A method to depyrogenate or inactivate viruses in a biological or pharmaceutical product comprising the steps of:

- 5           adsorbing said product onto a solid phase;  
          contacting said product adsorbed on said solid phase with a virus inactivating or depyrogenating agent;  
          separating the solid phase from the virus inactivating or depyrogenating agent;  
10          removing impurities and residual inactivating or depyrogenating agent from the solid phase; and  
          recovering the product from the solid phase.

2. The method of claim 1 wherein said solid phase is an ion exchanger resin.

15          3. The method of claim 1 wherein said solid phase is an affinity resin having attached thereto a ligand capable of adsorbing biological or pharmaceutical products.

4. The method of claim 3 wherein said ligand is an inhibitor, enzyme, coenzyme or hormone.

20          5. The method of claim 4 wherein said ligand is heparin.

6. The method of claim 1 wherein said solid phase is an affinity resin having attached thereto a  
25 monoclonal or polyclonal antibody.

7. The method of claim 6 wherein said antibody is antibody to Antihemophilic Factor antigen.

8. The method of claim 1 wherein said solid phase is controlled-pore glass beads or synthetic membrane  
30 adapted to produce ion exchange or affinity effect.



1           9. The method of claim 8 wherein the controlled-  
pore glass beads or the synthetic membrane are treated  
with an agent capable of adsorbing biological or  
5 pharmaceutical products, said agent is an inhibitor,  
enzyme, coenzyme, hormone, antibody or antigen.

10           10. The method of any of claims 1 to 9 wherein  
the virus inactivating or depyrogenating agent is an  
amphiphile, organic solvent, hypochlorite, beta  
propiolactone or mixtures thereof.

11. The method of claim 10 wherein the amphiphile  
is anionic, cationic, ampholytic or nonionic.

12. The method of claim 10 or 11 wherein said  
organic solvent is dimethyl ether, diethyl ether, ethyl  
propyl ether, methyl butyl-ether, methyl isopropyl ether,  
15 methyl isobutyl ether, chloroform, methanol, ethanol,  
propanol, butanol, or pentanol.

13. The method of any of claims 1 to 12 wherein  
the virus inactivating or depyrogenating agent is in a  
buffer solution having a pH of 5 to 9 and an ionic  
20 strength of 0.05 to 2.0M, the buffer solution containing  
a buffering agent selected from citrate, phosphate,  
borate, acetate, bicarbonate, succinate, maleate, phthalate,  
imidazole, trisamino methane, glycine, lysine, histidine, or  
mixtures thereof.

25           14. The method of any of claims 1 to 13  
wherein the virus inactivating or depyrogenating agent  
is in a gaseous or liquid form.

15           15. The method of any of claims 1 to 14 wherein  
the separating of the solid phase from the virus  
30 inactivating or depyrogenating agent is by filtration,  
centrifugation or by a column.

1           16. The method of any of claims 1 to 15  
wherein the removing of impurities and residual inactivating  
or depyrogenating agent is by washing said solid phase  
with a buffer solution, said buffer solution containing  
5 a buffering agent selected from citrate, phosphate,  
borate, acetate, bicarbonate, succinate, maleate, phthalate,  
imidazole, trisamino methane, glycine, lysine, or histidine,  
said buffer solution having a pH of 5 to 9 and an ionic  
strength of 0.05 to 2.0M.

10           17. The method of any of claims 1 to 16  
wherein the recovering the product from the solid phase  
is by elution with a buffer solution or desorbing agent,  
the buffering solution containing a buffering agent  
selected from citrate, phosphate borate, acetate,  
15 bicarbonate, succinate, maleate, phthalate, imidazole,  
trisamino methane, glycine, lysine, histidine or mixtures  
thereof and the desorbing agent is selected from sodium  
chloride, calcium chloride, magnesium chloride, epsilon  
amino caprioc acid or sodium citrate and mixtures thereof,  
20 the buffer solution having a pH of 5 to 9 and an ionic  
strength of 0.15 to 3.5M.

          18. The method of any of claims 1 to 17 wherein  
the biological or pharmaceutical product is a plasma  
protein, an enzyme, coenzyme, hormone, insulin, derived  
25 from human or animal placentae or prepared by recombinant  
DNA or gene splicing technique.

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